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Natural variation in fruit abscission-related traits in apple (*Malus*)

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Abstract Abscission or retention of ripening fruit is a major component of seed dispersal strategies and also has important implications for horticultural production. Abscission-related traits have generally not been targeted in breeding efforts, and their genetic bases remain mostly unknown. We evaluated 144 *Malus* accessions representing wild species, domestic cultivars, and hybrids for abscission-related traits. We found that seasonal timing of fruit abscission in wild species and hybrids showed a broad distribution similar to that seen for domestic cultivars, and that internal ethylene concentration at the time of abscission varied by over three orders of magnitude. Wild species, domestic cultivars, and hybrids all included representatives that showed abscission of fruit prior to substantial production of ethylene, as well as accessions that retained fruit for a significant period of time following ethylene production. For all accessions that retained fruit, fruit removal resulted in abscission of the pedicel, and exogenous ethylene promoted abscission, suggesting that the abscission zone was functional. Our results suggest important roles for mechanisms independent of fruit ethylene production in abscission.

Keywords *Malus* · Abscission · Fruit ripening · Ethylene · Natural diversity

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Introduction

During plant development, specific organs may undergo programmed separation from the main plant body, a process called abscission (Osborne 1989). Abscission plays crucial roles in the health and reproductive success of plants. For example, shedding of senescent leaves facilitates the recycling of mineral nutrients, abscission of floral organs after pollination allows for a focus of energy on reproduction, and dropping of diseased or infected organs reduces the spread of disease (Addicott 1982). Abscission of ripening fruits and mature seeds is an important process contributing to seed dispersal (Addicott 1982).

Organ separation typically occurs in a predetermined position, called the abscission zone. The abscission zone may differentiate very early or relatively late in the development of the organ, and is characterized by a few layers of small, densely cytoplasmic cells, generally arranged transversely to the organ axis (Addicott 1982; Osborne 1989; Sexton and Roberts 1982; Stösser et al. 1969a, b; Webster 1968). During initiation of abscission, these separation layer cells expand and may divide. Subsequently, secretion

of hydrolytic enzymes, increased peroxidase activity, and loss of calcium and pectin from the wall of separation layer cells presumably lead to the dissolution of the pectin-rich middle lamella, weakening the cell wall and allowing disintegration of abscission zone tissues (Addicott 1982; Morre 1968; Osborne 1989; Rasmussen and Bukovac 1969; Stösser et al. 1969b; Wittenbach and Bukovac 1975). Cells basal to the separation layers may undergo a process of transdifferentiation to form a protective layer continuous with the periderm of the stem (Addicott 1982). The vasculature, which passes through the separation layers, may not always participate in abscission (Stösser et al. 1969a) thus providing a final connection to the main plant body that can be broken by physical force.

Various environmental and developmental signals have been shown to induce abscission by influencing the ratio between auxins and ethylene within the organ and adjacent abscission zone cells (Addicott 1982; Brown 1997; Roberts et al. 2002; Taylor and Whitelaw 2001). According to a widely accepted model developed with leaf explants, loss of basipetal flow of auxin through the abscission zone, for example during leaf senescence, activates abscission by derepressing sensitivity of separation layer cells to ethylene (Abeles and Rubinstein 1964; Addicott 1982; Osborne 1989; Sexton 1995). Thus a balance between auxin and ethylene signaling, rather than absolute levels of the hormones, seems to be the predominant effector of abscission. The extent to which this leaf model can be applied to other organs, such as fruit, is not clear. However, it is known that developing fruit constitute a strong source of auxin, which is transported basipetally across the separation layers of the fruit pedicel, and that loss of auxin transport is associated with fruit abscission (Dražeta et al. 2004; Else et al. 2004). In plants such as apple that naturally adjust crop load to meet physiological capacity, auxin transport from dominant young fruit may repress transport from dominated fruit (Bangerth 2000). Dominated fruit may initiate abscission apparently in the absence of high levels of ethylene production, perhaps reflecting a predominant role for auxin in mediating abscission of young fruit (Bangerth 2000).

The interactive contributions of auxin and ethylene signaling to abscission of mature fruit have not been extensively studied. In many fruits, ripening is accompanied by the production of significant amounts of ethylene (Brady and Speirs 1991; Reid 1985). Transgenic melon expected to suppress expression of a fruit ripening-related *ACC OXIDASE* gene, and thus accumulation of fruit ethylene, showed loss of abscission (Ayub et al. 1996), and in domestic apple (*Malus domestica* Borkh.), blocking climacteric ethylene production in the fruit through the use of the ethylene agonist 1-methylcyclopropene was associated with delayed abscission (Sato et al. 2004). Walsh (1977) observed that, for three domestic apple cultivars, abscission was preceded by the accumulation of high levels of ethylene in the fruit. Although such experiments suggest that fruit-produced ethylene can promote abscission, whether this ethylene acts directly or indirectly, and the exact mechanism of this effect, remain unknown. Maturity in many fruits that naturally abscise is not associated with high levels of ethylene production [e.g., sour cherry (Wittenbach and Bukovac 1974)], suggesting either that low levels are sufficient to promote abscission or that natural abscission can occur independently of ethylene. It is well known that exogenous ethylene accelerates abscission of ripening fruit in a variety of fruit species, even those that do not produce high levels of endogenous ethylene (Abeles et al. 1992; Brady and Speirs 1991). As shown for a leaf explant abscission model, increased levels of ethylene in the fruit may reduce basipetal transport of auxin to the abscission zone, at least in part by decreasing auxin transport capacity (Beyer and Morgan 1971; Riov and Goren 1979; Suttle 1988). This mechanism may be superimposed on the endogenous decrease in auxin synthesis in the fruit associated with maturity, a phenomenon that may itself derepress ethylene generation (Abeles and Rubinstein 1964).

Fruit abscission in advance of harvest (pre-harvest drop) is a considerable production problem for many fruit crops, especially apple and pear (*Pyrus communis* L.). In commercial apple, the timing of natural fruit drop, relative to the optimal commercial harvest date, shows a large degree of variability. Some cultivars, such as McIntosh, are especially prone to preharvest drop. The genetic basis of this variability remains obscure. Sato et al. (2004) found that several apple cultivars homozygous for the dysfunctional *ACS1-2* allele of the *ACC SYNTHASE 1* gene, which is important for climacteric ethylene accumulation in apple (Costa et al. 2005; Harada et al. 2000), showed relatively low degrees of pre-harvest drop, and that

homozygosity of *ACS1-2* was associated with that low preharvest drop in a small segregating population. This supports the pharmacological evidence implicating climacteric ethylene in promoting abscission, and suggests that *ACS1* allelotype is an important contributor. However, this study also found that cultivars homozygous for the wild-type *ACS1-1* allele nevertheless can show a range of abscission behavior, ranging from nearly complete retention of fruit to nearly complete pre-harvest drop, revealing the importance of additional factors (Sato et al. 2004). This analysis was confounded by the fact that the optimal timing of commercial harvest is not based solely on maturity indicators, but also on storage potential, which may decline with a delay in harvest. Thus, varieties with greater capacity for storage may be harvested at a more advanced stage of maturity. Accordingly, internal ethylene concentration (IEC) of apple fruit has been found to vary dramatically among cultivars at the optimal time of commercial harvest (Chu 1988).

Although the relationships between the fruit ethylene production and abscission have been documented for a few domestic apple cultivars (Walsh 1977), there has been no analysis of this phenomenon at a large scale or including wild apple species. In tomato, variability in timing of abscission relative to climacteric ethylene production has been recognized among species (Grumet et al. 1981). Although the genetic basis of natural variation of this response in tomato has not been extensively studied, it is known that ethylene-independent fruit retention in tomato can be indirectly conferred by loss of function of *JOINTLESS*, a gene required for development of the abscission zone (Butler 1936). The potential influences of ethylene sensitivity, controlled largely by the availability of ethylene receptors (Klee 2001), in abscission have not been extensively studied in any fruit.

Retention of ripe fruit would be expected to confer considerable advantages for current production regimes, and would be critical for potential mechanical harvesting of apples. However, this trait has generally not been targeted in breeding efforts. Interestingly, the genus *Malus* includes many wild species that are anecdotally known to retain mature fruit, especially the small-fruited species commonly referred to as crabapples (Fiala 1994). A representation of *Malus* species and genotypes is maintained at the USDA-ARS Plant Genetic Resources Unit in Geneva, NY. This reference collection includes 28 wild *Malus* species and over 1000 *M. domestica* cultivars originating from throughout the northern hemisphere. A core subset considered to represent the diversity of the entire collection is maintained at the Geneva site, allowing for efficient evaluation of important traits relevant to industrial production (Hokanson et al. 2001; Kresovich et al. 1995). To better characterize variability in endogenous timing of fruit abscission, and help define the influence of fruit-produced ethylene in abscission of mature fruit, we analyzed variation in abscission-related responses among these accessions. Specifically, we assessed (1) seasonal timing of natural fruit abscission, (2) endogenous ethylene production at the time of abscission, (3) pedicel abscission in response to fruit removal, and (4) abscission of fruit in response to exogenous ethylene.

Materials and methods

Plant material

The *Malus* Germplasm Collection is maintained at the United States Department of Agriculture-Agricultural Research Service (ARS) Plant Genetic Resources Unit in Geneva, NY. We targeted for evaluation a subset of accessions previously determined to represent much of the diversity of the entire collection [the apple 'Core Collection' (Hokanson et al. 2001; Kresovich et al. 1995)] as well as accessions previously noted by USDA staff as exhibiting either premature fruit drop or fruit retention into the winter. Plants were five to ten years old, budded on M7 or E7 semi-dwarfing rootstocks, and managed in accordance with commercial practice only for insect or microbial pests. Only healthy and vigorous trees were selected for evaluation. Species, hybrid and cultivar nomenclature exactly followed ARS assignments.

Analysis of fruit abscission

For each accession, we determined the peak timing of fruit abscission by counting the number of naturally abscised fruit at two-week intervals, beginning August 27, 2006 when appreciable fruit abscission in some accessions was first noticed, and ending November 10, 2006, when trees were mostly defoliated. Peak abscis-

sion was defined as the observation date when at least 15% of fruit initially recorded for the accession had abscised. In all cases, nearly all remaining fruit abscised by the subsequent observation date (not shown). Accessions that showed less than 15% abscission of initial fruit at the final observation date were defined as non-abscising. Of accessions classified as non-abscising in 2006, all but six also showed less than 15% fruit abscission when evaluated at an equivalent date in 2007, with the remaining six accessions showing 50% or less abscission in 2007. For each abscising accession, on the date defined as peak abscission, 20 fruit were selected that abscised when subjected to gentle force. All collected fruit separated from the branch at the apparent pedicel/branch abscission zone, and showed turgid, undamaged pedicels. On the final observation date, fruit from non-abscising accessions were removed from the tree, leaving the pedicel attached to the fruit. Fruit were maintained under laboratory conditions for 24 h before analyses.

Measurement of internal ethylene concentration (IEC), firmness and starch

All determinations of IEC, firmness, and starch were based on measurements of at least five fruit of each accession. For IEC measurement, 1 ml of internal gas was withdrawn from fruit submerged in water under a vacuum (Beyer and Morgan 1970), and analyzed by gas chromatography using a Carle Series 400 AGC; Hach Co., Loveland, CO) and certified ethylene standard (Matheson Gas Products, Chicago, IL). Flesh firmness was determined using an Effigy FT-327 penetrometer (Effegi, Alfonsine, Italy) with an 11-mm diameter probe. Starch content was evaluated by rating stain intensity after dipping transverse sections into an iodide solution (5 mM potassium iodide, 17 mM iodine), with a visual scale of 1 (intense staining; highest starch content) to 8 (no staining; lowest starch content) using the Cornell Generic Starch Chart.

MdACS1 genotyping

MdACS1 allelotype was evaluated by the PCR using oligonucleotide primers *MdACS1-F* (5′-GGTAATTG GAGTAATGAACTGAGCA-3′) and *MdACS1-R* (5′-T CACTATTTGCTTGGACTGGGAAGT-3′) that flank the transposon insertion found in *MdACS1-2*, as described by Sunako et al. 1999.

Evaluation of pedicel abscission and ethephon-promoted abscission

This experiment was carried out in early July, 2007 approximately 80 days after full bloom for the standard cv. Gala. For each accession evaluated, 30 fruit were labeled on each of two branches. On one of the two branches, the pedicel was severed midway between the fruit and the branch to induce abscission. Abscission was monitored daily by applying a gentle force on the defruited pedicel. On the remaining branch, marked fruit were evaluated for natural abscission (control). A biological replicate, offset by two days, was carried out using a separate branch, or branch of a separate tree. Abscission zone morphology was evaluated on pedicels of fruit attached to the tree, without the aid of microscopy.

Analysis of promotion of fruit abscission with ethephon was carried out in mid-late September, 2007. A subset of non-abscising accessions identified in 2006 was targeted for analysis. For each accession, branches with similar fruit load were tagged as either experimental or control. For each of the experimental treatments, 2-chloroethylphosphonic acid (Ethephon, 600 µl/l active ingredient) was applied with 0.1% Silwet S-77 as a foliar spray. The control branch was treated with 0.1% Silwet only. The replicate treatment was offset by one day. Fruit IEC was determined as described above. Fruit abscission was quantified by counting the number of abscised fruit at defined intervals following treatment, and expressed as percentage of initial number of fruit recorded for the accession.

Results

Seasonal timing of fruit abscission

To document variation in the seasonal timing of fruit abscission among wild *Malus* species, *M. domestica* cultivars, and hybrids, we examined 144 diverse accessions at defined intervals during the period of natural fruit abscission. These included 53 accessions representing 28 wild species, 61 *M. domestica* cultivars, and 30 hybrids (Table 1 and not shown). We found that the seasonal timing of fruit abscission was similarly and broadly distributed across observation dates for representatives of wild *Malus* species, *M. domestica* cultivars, and hybrids, and that all three

Table 1 Number of wild species, domestic cultivars, and hybrids used in this study

Wild species	53
angustifolia	1
asiatica	3
atrosanguinea	1
baccata	4
bhutanica	1
coronaria	5
halliana	1
hupehensis	1
ioensis	3
kirghisorum	2
mandshurica	1
micromalus	1
prunifolia	3
rockii	1
sieboldii	2
sieversii	5
sylvestris	2
turesii	1
× *arnoldiana*	1
× *dawsoniana*	1
× *hartwigii*	1
× *magdeburgensis*	1
× *platycarpa*	1
× *robusta*	4
× *scheideckeri*	1
× *soulardii*	2
× *sublobata*	1
yunnanensis	2
Domestic cultivars (*M. domestica*)	61
Hybrids	30

groups included non-abscising accessions. However, accessions showing abscission at the earliest two observation dates were mainly *M. domestica* cultivars (25 of 33 accessions), whereas non-abscising accessions were predominantly wild *Malus* species or hybrids (36 of 49 accessions) (Fig. 1a). We then evaluated the potential relationship between seasonal timing of fruit abscission and fruit size. We categorized fruit into three classes: >100 g, 30 g–100 g, and <30 g, and identified representatives of each fruit size class that exhibited abscission at each observation date. Small-fruited accessions, nearly all wild species and hybrids, were overrepresented among the non-abscising class (31 out of 49 accessions) (Fig. 1b). However, we observed 23 small-fruited accessions that abscised, and 14 large-fruited accessions, nearly all domestic cultivars, that did not abscise (Fig. 1b). This documents an association between fruit retention and non-domestic, small-fruited genotypes, lack of abscission among a small number of domestic cultivars, and the existence of alleles specifying early season fruit drop among wild species.

Variation in fruit ethylene concentration at abscission

To help evaluate a potential role for fruit-produced ethylene in abscission, we measured internal ethylene concentration (IEC) of fruit of each accession harvested at the date of peak natural abscission. For each accession, readily abscising fruit were removed from the tree, briefly allowed to equilibrate to the laboratory environment (Sfakiotakis and Dilley 1973), and assayed for IEC. We found that the IEC in abscising fruit among different accessions varied by greater than three orders of magnitude, from ~0.03 µl/l to 900 µl/l (Fig. 2 and not shown). Multiple fruit from single accessions generally showed low variability in IEC (standard error ~15% of mean values) suggesting the observed variability reflected true tree-to-tree differences (not shown). Those accessions showing the lowest IEC values in abscising fruit (≤0.5 µl/l) included nine accessions (eight domestic and one wild) that also exhibited high starch content, suggesting that the ripening program had not significantly progressed in these accessions (Table 2). We also evaluated IEC in fruit from non-abscising accessions. Unblemished fruit were removed from the tree in early November, allowed to briefly equilibrate to the laboratory environment, and assayed for IEC under the same conditions as for naturally abscising fruit. Surprisingly, the range of IEC from non-abscising fruit was similar to that observed in abscising fruit (~0.07 µl/l to 580 µl/l), although accessions with non-abscising fruit were underrepresented in the group of accessions with highest IEC values (≥10 µl/l) (18 of 78 accessions) (Fig. 2). All non-abscising accessions with high IEC values (≥10 µl/l) showed relatively low starch content (starch index ≥6), and at least a subset of these also exhibited low flesh firmness values (≤~90 N) and fully developed skin ground color (Table 2 and not shown) suggesting that the ripening program was progressing. This subset included *M. prunifolia* and *M.* × *asiatica* (=*M.*

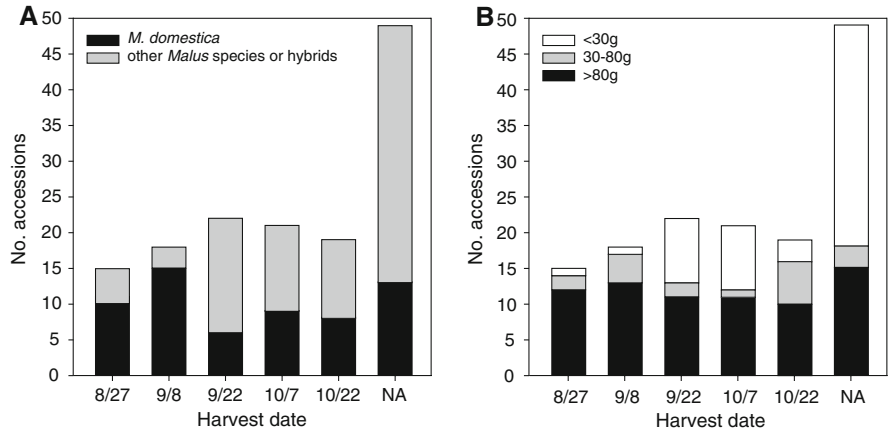

Fig. 1 Frequency distribution of accessions evaluated for peak abscission date relative to species (**a**) and fruit weight (**b**) (NA, non-abscising)

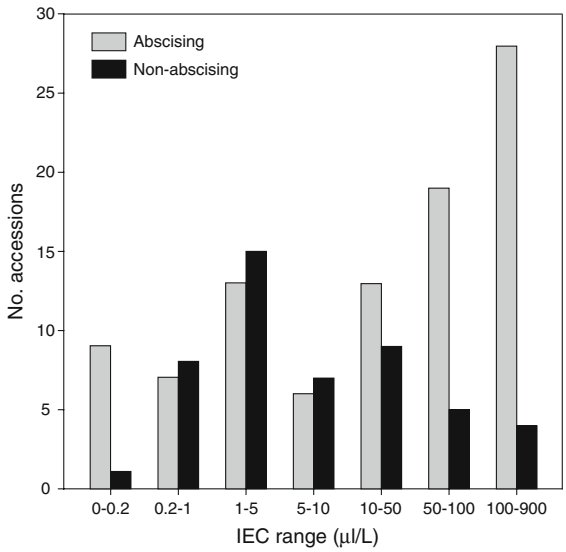

Fig. 2 Frequency distribution of accessions evaluated for internal ethylene concentration (IEC) at peak abscission date (abscising accessions) or final sampling date (non-abscising accessions)

prunifolia × *M. sieversii*; Luby 2003), four domestic cultivars, and two hybrids (Table 2).

It was previously reported that absence of significant preharvest drop among *M. domestica* cultivars was associated with homozygosity of the dysfunctional *MdACS1-2* allele (Sato et al. 2004; see above). To further understand the variation in seasonal timing of abscission and relationship between abscission and fruit IEC, we determined the *MdACS1* allelotype for the studied accessions. The *ACS1-1/1* allelotype was identified in >70% of accessions, including 82 wild species, 20 hybrids, and 37 domestic cultivars, whereas only ~9% of accessions (6 hybrids and 6 domestic cutivars) exhibited the *ACS1-2/2* allelotype (not shown). We found that each allelic group (*MdACS1-1/1, -1/2,* or *-2/2)* contained both abscising and non-abscising accessions (Fig. 3). The *MdACS1-1/1* allelotype was overrepresented among accessions showing the earliest natural abscission (August 27; 12 of 13 accessions), whereas the *MdACS1-2/2* allelotype was overrepresented among non-abscising accessions (7 of 49 accessions) (Fig. 3). This suggests that *MdACS1* allelotype is not only a possible determinant of the potential for preharvest drop, but also for the non-abscising character.

To evaluate the relationship between timing of abscission and climacteric-associated ethylene production, and to determine if lack of capacity for climacteric ethylene production may have contributed to the low IEC in some accessions, we measured IEC in harvested fruit after storage in the laboratory environment for 14 days (Gussman et al. 1993; Sfakiotakis and Dilley 1973). Of 14 abscising accessions evaluated, 13 showed a striking (>500-fold) increase in IEC during this period (Table 2). These accessions were naturally abscising, revealing that autocatalytic ethylene production followed rather than preceded natural fruit drop in these accessions.

Abscission in response to fruit removal and exogenous ethylene

These experiments identified 18 accessions that failed to abscise yet produced high levels of ethylene in the fruit. These were not obviously distinguished from the entire population in terms of *ACS1* allelotype, IEC at harvest, or fruit weight (not shown). All of the

Table 2 Selected accessions showing extremes of IEC in abscising or non-abscising fruit

Accession	Species	Cultivar	*ACS1* allelotype	IEC (μl/l) 1 day after harvest	IEC (μl/l) 14 days after harvest	Firmness (N)	Starch index (1 = most; 8 = least)
Accessions showing low IEC in abscising fruit							
589071	*domestica*		*ACS1-1/2*	0.03	72.3	86.9	1.0
588943	*domestica*	Liberty	*ACS1-1/1*	0.10	593.0	121.0	1.0
158731	*domestica*	Bramtot	*ACS1-1/2*	0.24	208.8	107.8	1.0
199532	*domestica*	Toyo	*ACS1-1/1*	0.27	212.2	92.8	1.0
483257	*domestica*	Reinette Simirenko	*ACS1-1/1*	0.30	286.9	83.4	1.0
483254	× *dawsoniana*		*ACS1-1/1*	0.04	246.6	117.9	1.2
588848	*domestica*	Cortland	*ACS1-1/1*	0.45	972.5	90.7	1.6
134808	*domestica*		*ACS1-1/2*	0.08	635.1	107.7	2.0
589511	*domestica*	Severny Sinap K-21.39	*ACS1-1/1*	0.12	510.6	80.2	2.0
588998	*domestica*	Marshall McIntosh	*ACS1-1/1*	0.13	1063.7	81.6	5.0
589780	*hybrid*	PRI 384-1	*ACS1-1/1*	0.07	554.0	73.9	6.2
280401	*domestica*	Ein Shemer	*ACS1-1/2*	0.37	625.9	79.7	6.6
588866	*hybrid*	Kerr	*ACS1-1/1*	0.12	6.2	92.1	8.0
589391	× *soulardii*		*ACS1-1/1*	0.14	167.3	>133.5	8.0

Accession	Species	Cultivar	*ACS1* allelotype	IEC 1 day after harvest	Firmness	Starch index
Accessions showing high IEC and low starch content in non-abscising fruit						
588747	*domestica*	Florina	*ACS1-1/2*	20.3	80.4	7.4
589819	*hybrid*	PRI 2050-2	*ACS1-2/2*	25.7	76.5	7.0
589790	*hybrid*	PRI 1484-1	*ACS1-2/2*	68.1	68.3	6.0
162722	*domestica*	Damelot	*ACS1-1/1*	80.5	72.3	8.0
199525	*domestica*	Amanishiki	*ACS1-1/2*	99.9	90.5	6.2
589539	*domestica*	Zlatna Resistenta	*ACS1-1/2*	157.7	32.5	8.0
589877	*asiatica*		*ACS1-1/2*	218.0	59.1	8.0
589389	*prunifolia*	Macrocarpa	*ACS1-1/1*	402.1	52.4	8.0
589874	*asiatica*		*ACS1-1/2*	583.0	111.3	6.0

non-abscising accessions, including these, exhibited classical abscission zone morphology. To evaluate the functionality of the abscission zone in these accessions, we determined the abscission response of the fruit pedicel when fruit was removed from field-grown plants by cutting the pedicel halfway between the branch and fruit (Barlow 1950). We previously observed that this treatment invariably resulted in abscission of the remaining pedicel stub at the natural abscission zone within 5–8 days, when carried out with the Golden Delicious cultivar approximately 80 days following full bloom (unpublished data). To interpret the results, we applied this analysis to nearly the entire population. For the experiments reported here, a marked subset of fruit on each tree was removed, and the timing of abscission of the remaining pedicel stubs was noted at a daily interval.

Interestingly, all of the 122 accessions used in this experiment showed abscission of pedicel stubs, with the median timing of abscission among accessions ranging from 3 to 17 days. We identified a subset of 42 accessions that showed relatively rapid and synchronous abscission of all treated pedicels, beginning ~3 days after treatment, and with >50% or >95% of pedicels abscising within 24 or 48 h, respectively, thereafter (Table 3). Abscission of pedicels for the remaining accessions was less rapid and less synchronous, with a subset of 24 accessions showing abscission only after ~5 days, and retention of >50% of pedicels for at least five days thereafter. The

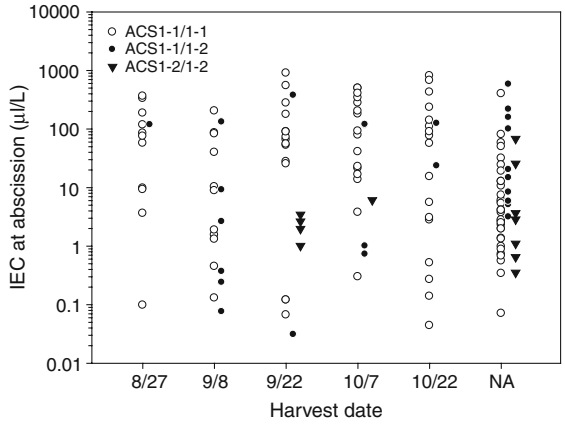

Fig. 3 Relationship between harvest date, IEC at abscission, and *ACS1* allelotype for studied accessions

synchronous-abscising subset was disproportionally lacking in domestic cultivars, large-fruited accessions, and/or genotypes heterozygous or homozygous for *ACS1–2* ($P < 0.05$, Fisher's Exact Test; Table 3). Neither the non-abscising accessions producing high levels of ethylene, nor non-abscising accessions considered as a whole, were distinguished from the remainder of accessions in terms of the abscission behavior of the pedicel following cutting.

As an alternative approach to analyze functionality of the abscission zone, we subjected 24 non-abscising accessions to treatment with 2-chloroethylphosphonic acid (Ethephon), which is metabolized by plant tissues to produce free ethylene. Branches within the same tree were either subject to a foliar spray, or used as mock-treated controls. Of these accessions, 15 showed abscission of >50% of fruit within 12 days of treatment, and all but three showed abscission that was markedly greater than that of the control branches (Table 4). The remaining three accessions showed abscission of <2% of fruit after ethephon treatment; however, fruit from these did not show substantial increases in IEC, suggesting that the ethephon treatment was ineffective (Table 4).

Discussion

In this study, variation in abscission-related traits was observed among *Malus* accessions representing the breadth of genetic diversity found in domesticated varieties and in the *Malus* genus. This information is useful both for understanding the underlying biology of these traits and for the more applied goal of developing genotypes with abscission characteristics better suited to modern apple production approaches.

We documented the range in seasonal timing of natural abscission for domestic and non-domestic accessions, and the occurrence of accessions lacking fruit abscission. For domestic cultivars, seasonal distribution of abscission is most likely influenced by the broad range in seasonal timing of fruit maturation, a trait perhaps subject to selection during domestication. However, we found a similar range of seasonal timing of abscission among wild species. In this study, replicate observations of seasonal timing of abscission could not be made for most accessions, because only a single specimen was available during the duration of the study. Possibly, some tree-to-tree differences observed were influenced by the physiological status of individual trees, rather than strictly by genotype. However, none of the trees used in this study displayed visible signs of stress. Neither did we observe separation of fruit from the tree independently of the visible abscission zone, a phenomenon that has been connected with abscission related to tissue damage (Walsh 1977).

We also evaluated the tendency for natural abscission in relation to fruit size. It is well known that many crabapple-type *Malus* genotypes retain fruit into the winter season. Potentially, retention of ripened fruit in small-fruited genotypes is an adaptation that facilitates access and seed dispersal by frugiverous birds (Harris et al. 2002). In contrast, domestication of large-fruited genotypes may have favored those that readily dropped ripe fruit, in order to facilitate collection from the naturally large trees. In this study, we documented the anecdotal observations that lack of abscission is not absolutely coupled to small fruit size. We identified 14 accessions, ten domestic cultivars and four hybrids, that exhibited relatively large (>100 g) fruit and that did not show abscission. Thus, alleles governing this trait may be readily exploited in the development of new commercial varieties.

A trivial explanation for the lack of abscission identified in some accessions in this study is that these genotypes may be adapted to longer growing seasons, and fail to initiate the abscission process at the Geneva site before the onset of dormancy. While this is possibly the case for some of the accessions, we

Table 3 Accessions showing extremes of pedicel abscission

Accession	Species	Cultivar	Days to first abscission	Days to 50% abscission	Days to 95% abscission	Fruit weight (g)	*ACS1* Allelotype
Accessions showing relatively rapid and synchronous pedicel abscission							
589765	*angustifolia*		3	4	4	12.3	*ACS1-1/1*
589877	*asiatica*		4	4	5	48.4	*ACS1-1/2*
136488	*atrosanguinea*		3	3	3	7.5	*ACS1-1/1*
588907	*baccata*	Himalaica	3	3	3	1.8	*ACS1-1/1*
322713	*baccata*	Mandshurica	3	3	4	5.6	*ACS1-1/1*
483259	*baccata*	Genvina	3	3	5	9.5	*ACS1-1/1*
590062	*bhutanica*		3	4	5	1.5	*ACS1-1/1*
589987	*coronaria*		3	3	4	20.9	*ACS1-1/1*
589983	*coronaria*		3	3	4	25.6	*ACS1-1/1*
588849	*domestica*	Russian	4	5	6	22.1	*ACS1-1/1*
589478	*domestica*	Novosibirski Sweet	4	4	5	45.6	*ACS1-1/1*
589053	*domestica*	Lady	4	5	6	75.9	*ACS1-1/1*
588838	*domestica*	Nova Easygro	4	5	6	107.0	*ACS1-1/1*
589913	*domestica*	Dorsett Golden	5	6	6	110.3	*ACS1-1/1*
589486	*domestica*	Murray	4	4	5	175.5	*ACS1-1/1*
588992	*hybrid*	White Angel	3	4	4	2.5	*ACS1-1/1*
589819	*hybrid*	PRI 2050-2	5	6	7	198.9	*ACS1-2/2*
589820	*hybrid*	Paririe Fire	3	4	5	1.3	*ACS1-1/1*
589959	*hybrid*	MA #8	3	4	4	2.0	*ACS1-1/1*
589510	*hybrid*	Garry	3	4	4	2.5	*ACS1-1/1*
588824	*hybrid*	Almey	3	4	4	3.8	*ACS1-1/1*
589250	*hybrid*	Red Jacket	3	3	4	8.0	*ACS1-1/1*
588870	*hybrid*	Dolgo	3	3	4	13.9	*ACS1-1/1*
589775	*hybrid*	PRI 2382-1	5	6	7	92.8	*ACS1-1/1*
588883	*hybrid*	Demir	4	5	6	109.0	*ACS1-1/1*
590016	*ioensis*		3	4	5	14.6	*ACS1-1/1*
590004	*ionesis*		3	4	5	10.6	*ACS1-1/1*
613855	*kirghisorum*		3	4	5	43.0	*ACS1-1/1*
589832	*prunifolia*	Xanthocarpa	3	3	4	4.3	*ACS1-1/1*
589421	*rockii*		3	4	5	2.8	*ACS1-1/1*
613932	*sieboldii*		5	5	6	0.4	*ACS1-1/1*
613806	*sieboldii*		3	3	3	0.4	*ACS1-1/1*
594104	*sieversii*		4	5	5	26.8	*ACS1-1/1*
589008	*turesii*		3	3	4	1.7	*ACS1-1/1*
588757	× *hartwigii*	GMAL52	4	4	5	1.9	*ACS1-1/1*
588959	× *magdeburgensis*		6	7	8	0.8	*ACS1-1/1*
589415	× *platycarpa*	Hoopesii	3	3	3	34.5	*ACS1-1/1*
588825	× *robusta*	Robusta 5	3	3	4	3.1	*ACS1-1/1*
589383	× *robusta*	Persicifolia	3	4	5	3.9	*ACS1-1/1*
589418	× *scheideckeri*		3	3	4	3.1	*ACS1-1/1*
588922	× *sublobata*	Yellow Autumn Crab	3	3	4	12.3	*ACS1-1/1*
589253	*yunnanensis*	Carmine crab	4	5	6	0.8	*ACS1-1/1*

Table 3 continued

Accession	Species	Cultivar	Days to first abscission	Days to 50% abscission	Days to 95% abscission	Fruit weight (g)	*ACS1* Allelotype
Accessions showing relatively delayed and asynchronous pedicel abscission							
589874	*asiatica*		9	17	19	44.2	*ACS1-1/2*
589991	*coronaria*		4	8	11	15.7	*ACS1-1/1*
588747	*domestica*	Florina	5	9	11	136.3	*ACS1-1/2*
392303	*domestica*	Gala	4	8	9	111.8	*ACS1-2/2*
502248	*domestica*	Lady Williams	4	9	12	120.8	*ACS1-1/2*
307380	*domestica*	Nubeena	4	10	12	131.1	*ACS1-1/1*
347268	*domestica*	Kokko strain 2	6	10	13	143.4	*ACS1-2/2*
588841	*domestica*	Idared	5	9	13	149.2	*ACS1-1/2*
307382	*domestica*	Sturmer Pippin	7	11	13	152.9	*ACS1-1/1*
588842	*domestica*	Empire	4	8	9	155.9	*ACS1-1/1*
588850	*domestica*	Rome Beauty Law	7	11	13	158.2	*ACS1-1/2*
589894	*domestica*	Keepsake	6	11	14	164.6	*ACS1-1/2*
588848	*domestica*	Cortland	6	11	15	177.9	*ACS1-1/1*
589294	*domestica*	Fallawater	5	9	10	206.9	*ACS1-1/2*
589553	*domestica*	Mantuanskoye	5	9	10	211.2	*ACS1-1/1*
589893	*domestica*	Holly	4	8	10	270.5	*ACS1-2/2*
588981	*domestica*	Mollie's Delicious	5	9	11	334.3	*ACS1-1/1*
589790	*hybrid*	PRI 1484-1	5	11	13	137.0	*ACS1-2/2*
589274	*hybrid*	Masek	5	11	16	6.6	*ACS1-1/1*
590085	*hybrid*	PRI 1176-1	5	9	9	145.4	*ACS1-1/1*
590009	*ioensis*		5	10	12	15.2	*ACS1-1/1*
613845	*prunifolia*		5	10	13	24.6	*ACS1-1/2*
588993	× *robusta*		5	11	15	8.9	*ACS1-1/1*
589003	× *robusta*	Korea	5	14	20	11.2	*ACS1-1/1*

noted that many of the non-abscising accessions showed physiological characteristics that are typically associated with ripening in domestic cultivars, including color development, loss of starch, and loss of firmness, and/or also exhibited relatively high levels of internal ethylene (Table 2 and not shown; see below). For these, abscission is apparently unlinked from ripening and/or ethylene production.

Previous studies have shown that internal ethylene concentration can vary strikingly among domestic varieties at the date of optimal commercial harvest, a benchmark based both on fruit maturity and storage potential (e.g., Chu 1988). In this study, we analyzed internal ethylene concentration at the time of abscission, or for non-abscising accessions, at a time late in the season when plants were progressing into dormancy. Blanpied (1972) observed substantial natural abscission in cv. Golden Delicious and McIntosh preceding climacteric ethylene production, suggesting that for these varieties, high levels of fruit ethylene are not required for abscission. There have been no previous large-scale studies to examine genotypic differences in ethylene production in relation to fruit abscission. Here, we found that internal ethylene content of abscising fruit varied substantially among both domestic and non-domestic accessions.

We identified numerous accessions that showed natural abscission even when the IEC was low. These included Marshall McIntosh, a skin color variant of McIntosh. In our study, McIntosh showed only moderate starch content and relatively low flesh firmness at abscission. This suggests that ripening was in progress, and supports both the observation of Blanpied (1972) that this cultivar abscises in advance of significant ethylene production, and anecdotal observations that this cultivar is prone to premature abscission. We

Table 4 Internal ethylene concentration (IEC) and abscission in response to Ethephon

Accession	Species	Cultivar	IEC of treated fruit (μl/l)[a]	IEC of control fruit (μl/l)	Abscission of treated fruit (% initial)	Abscission of control fruit (% initial)
483259	*baccata*	Genvina	11.46	3.64	100.0	0.0
589393	*hybrid*		12.26	2.16	100.0	0.0
588757	× *hartwigii*	GMAL52	16.07	3.27	100.0	6.9
589959	*hybrid*	MA #8	21.08	3.85	100.0	8.4
589820	*hybrid*	Paririe Fire	50.49	3.75	93.1	0.0
306320	*domestica*	Crittenden	35.96	2.32	91.0	0.0
588907	*baccata*	Himalaica	9.91	2.12	87.1	0.0
588825	× *robusta*	Robusta 5	17.85	1.35	85.5	0.0
589421	*rockii*		47.14	3.20	84.7	0.0
590062	*bhutanica*		11.63	3.30	83.3	4.3
613835	*asiatica*		5.96	4.27	80.1	8.9
589008	*turesii*		35.25	31.23	68.8	6.3
588993	× *robusta*		36.75	7.89	67.2	0.0
589498	*domestica*	Dab 100	5.36	1.03	64.3	2.6
588992	*hybrid*	White Angel	58.57	12.12	57.4	0.0
594092	*micromalus*		7.83	3.60	34.7	0.0
589877	*asiatica*		8.50	1.12	31.3	0.0
589003	× *robusta*	Korea	30.89	27.71	29.0	5.0
613932	*sieboldii*		6.76	0.86	24.8	0.0
613834	*hupehensis*		7.31	0.75	20.9	0.0
589874	*asiatica*		5.46	1.88	18.5	0.0
589274	*hybrid*	Masek	4.91	2.14	1.9	0.0
588856	*hybrid*	Ottawa 8	3.47	1.22	1.4	0.0
271831	*hybrid*	Vimorin	4.11	1.64	0.0	0.0

[a] Mean value of measurements for two replicates 3d, 6d, 9d, and 12d following treatment

noted that this accession was distinguished among all of the evaluated accessions by an extremely short pedicel, a trait that may lead to substantial physical force on the abscission zone as the fruit enlarges and becomes constrained by the branch or neighboring fruit. In other accessions that showed abscission at low IEC, ripening had apparently not significantly progressed, as evidenced by the high starch content. Interestingly, eight of the nine accessions that showed abscission in advance of apparent ripening are domesticated cultivars, and the ninth, *M.* × *dawsoniana*, is believed to have *domestica* parentage, suggesting that abscission in advance of ripening may have been subject to selection during domestication.

In contrast, we also identified accessions that did not show abscission, in spite of high levels of ethylene in the fruit. A potential explanation is that the abscission process was initiated, but was in an early stage, at the time of our measurements. However, this appears unlikely, since our analysis was carried out at a time during the season when trees were mostly defoliated and entering dormancy. In addition, none of the fruit from non-abscising accessions appeared to be nearing abscission, because significant force was required to remove the fruit at the apparent abscission zone when these were harvested (not shown). These accessions, which include four domestic cultivars and two hybrids with Golden Delicious parentage, are an attractive source of alleles conferring this trait for breeding of commercial varieties.

It is interesting to speculate on the determinants that might govern the apparent ethylene-independent retention of fruit seen in these accessions. All of the non-abscising accessions examined in this study showed an adjacent enlargement and constriction at the basal end of the pedicel, a characteristic of the abscission zone in cultivated apple. In addition, our experiments showed that abscission could be induced

either through removal of the immature fruit, or, for the subset of the accessions analyzed, by exposure to exogenous ethylene. This suggests that the natural fruit retention seen in these accessions was not due to a homeotic absence of the abscission zone, as seen in tomato mutants for the *JOINTLESS* gene. However, we cannot rule out the possibility that one or more of these accessions show more subtle defects in abscission zone function that precludes abscission of fruit under natural conditions. For example, incomplete separation layer development has previously been shown as a mechanism associated with loss of grain abscission in domesticated rice (Li et al. 2006). Anatomical comparisons of accessions showing extremes of abscission habits identified here may resolve this question. Another possibility is that the abscission process may be initiated, but remain ultimately ineffective for pedicel breakage. Some genotypes may lack effective production of one or more of the numerous enzymatic activities expected to be required for cell wall disassembly. Here, comparative expression analyses of selected abscission-associated genes among genotypes showing extremes in abscission behavior may be informative. Alternatively, some abscission zone tissues might not fully participate in abscission. For example, vascular tracheids and other cell types may have highly modified secondary walls that likely present a challenge for cell wall disassembly. These may persist after disintegration of other separation layer cell types, and effectively retain the fruit until broken by physical force. Especially for *Malus* genotypes that have small, lightweight fruits, even subtle variation in such cell types or numbers in the abscission zone could predispose the fruit to drop or retention. To explore this, a detailed study of abscission zone development among accessions is needed.

In the model plant *Arabidopsis thaliana*, a gene designated *IDA* is required for floral organ abscission. Interestingly, in *ida* mutant plants, organ removal force ultimately increases following a sharp decrease at the time of natural organ abscission (Butenko et al. 2003). This identifies a potentially conserved mechanism that may act antagonistically to cell disintegration, and in apple, may preclude efficient abscission in naturally non-abscising accessions.

In conclusion, we documented diversity in fruit-abscission-related traits among *Malus* accessions representing the breadth of genetic diversity seen in *Malus*. Our findings suggest that important mechanism(s) independent of fruit ethylene production act as determinants of natural abscission. Accessions showing phenotypic extremes in abscission-related traits can be developed as contrasting models to understand the biological bases of these traits, and as tools in genetic analyses for the mapping of genes that influence these traits.

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